COMPARISON OF ANTIMICROBIAL EFFECTS OF MTAD AND 1.3% SODIUM HYPOCHLORITE AGAINST ENTEROCOCCUS FAECALIS

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ABSTRACT

Aim: In this in vitro study, antimicrobial effects of MTAD (a mixture of tetracycline isomer, an acid and a detergent) and 1.3% sodium hypochlorite (NaOCl) against Enterococcus faecalis were evaluated.

Materials & Method: Forty extracted single-canal premolars were exposed for 4 weeks to infection with Enterococcus faecalis after initial preparation. Canal preparation with passive step back was done and then MTAD and 1.3% NaOCl protocols were performed for canal disinfection. Negative control group were autoclaved after preparation. Positive control group was prepared just with cleaning by distilled water. Groups of 3 and 4 were immersed in respected solutions for 5 min and after this step, for each tooth were rinsed in 2 ml of fresh BHI for 30 second. After one week incubation in BHI medium, the created turbidity was evaluated.

Results: Among 15 evaluated teeth in group 3 which rinsed with 1.3% NaOCl, 3 teeth were remained infected and all 15 evaluated teeth in group 3 which rinsed with MTAD were uninfected. Data were analyzed using Chi-square and Exact Fisher test. Statistical analysis was not shown any significant difference between two groups about turbidity (p>0.05).

Conclusion: MTAD solution has antibacterial effects against E. Feacalis and can be used as final rinsing after applying 1.3% NaOCl.

Key words: Antimicrobial, Enterococcus Faecalis, MTAD, Sodium Hypochlorite.

Introduction

Most of pulp and peri-apical diseases are resulted from direct or indirect interference of oral bacteria. This fact is shown a century ago and confirmed by developed immunological and bacteriological techniques. Based on current knowledge, most of changes in pulp and peri-apical tissue have bacterial origin similar to other infective process.[1,2]

In recent decades, Enterococcus faecalis gains specific attention in endodontic due to researches which conducted about its role in failure of root canal treatment. This microorganism is a Gram positive facultative anaerobic bacteria which resistant to treatment and is survived even with common rinsing.[3,4]

Because only with a mechanical instrument cannot be performed root canal treatment, one of the ways to combat with these microorganism is use of intra-canal irrigant and medicaments drugs.[5]

Agent which used for several years in different concentration as an endodontic irrigant is sodium hypochlorite (NaOCl) that its biggest advantage is resolving tissue and antimicrobial effects against most of microorganisms. But this agent also has some disadvantages such as bad taste, toxic effect, incomplete removing of smear layer and ineffectiveness on some bacterial strains.[6,7] One of the approximately new rinsing agent which used in dentistry is MTAD. This agent is a combination of tetracycline isomer (deoxycycline), one acid and one detergent.[5]

This agent has benefited from the penetrative property of deoxycycline and can penetrate into the dentinal tubules. With penetration to this tubules and more contact with bactria, it can induced more antibacterial effects.[8] Therefore, this study was performed with aim of comparing the antimicrobial effects of MTAD and 1.3% NaOCl.

Materials & Method

This study is an experimental cross-sectional study with population of extracted single-canal premolars human teeth. After selection of 40 single-canal premolars teeth and preparation and cleaning of external surfaces by a scaler, teeth were deepen in 1.3% NaOCl solution for 30 min and then exported to the saline solution for maintenance. Calcified cases or tooth with more than one canal were diagnosed by radiography and excluded from the study. Then by preparing of primary access, dental pulp was removed by Barbed Broache and rinsing with distilled water was performed. Canal length was measured by file no. 25 as tip to tip with apical foramen using light microscope (magnification ×10) and reducing 0.5 mm from them. After that, teeth were sterilized using autoclave and were deepen in brain heart infusion broth (BHI) as concentration as 1×108 cell/ml of E. faecalis for 4 weeks before instrumentation. To ensure the viability of bacteria during these 4 weeks, BHI media was refreshed every 2 days. Sampling from internal and external surfaces of teeth was done by fine paper point to inoculate in brain heart infusion broth (BHI) media. Dental infection was confirmed by growth of small colonies on the surface of BHI agar. Smear preparation and staining and then observing binary or chain of Gram positive cocci under microscope confirmed dental infection more and more. From this point on, glove and sterile tools were used for prevention of dental contamination. Teeth were randomly allocated into 4 groups and in each group, canals were prepared based on passive step back technique to an apical size 40 k-file (Mani, Japan) and using Gates Glidden (Mani, Japan) drills no. 1, 2, 3 for flaring as follow:

- **Group 1:** 5 teeth as positive control group which rinsed with distilled water during instrumentation.
- **Group 2:** 5 teeth of negative control group which rinsed with distilled water during instrumentation and then autoclaved. (Microbial sampling was done from negative control group to ensure that our methods were not induced infection).
- **Group 3:** 15 teeth in which 5 ml of 1.3% NaOCl solution (Tazh, Iran) was used during instrumentation and 5 ml of same solution as final rinsing. Then they were deepen in 1.3% NaOCl solution for 5 min (it was performed to eliminate possible contamination of external root surface).
- **Group 4:** 15 teeth in which 5 ml of 1.3% NaOCl solution was used during instrumentation and 5 ml of MTAD solution (Biopure, Dentsply Tulsa Dental, USA) as final rinsing. Then they were deepen in MTAD solution for 5 min.

It must note that needle gage 30 was used for all rinsing step of canals and time of using rinsing material through the canals were approximately 2-3 min. After 5 min deepening time in each respected solutions, each canal was dried with paper point separately and then were deepen in 2 ml of BHI broth and shake for 15 seconds for prevention of transferring of rinsing solution remnant to culture media. Then each teeth was transported to 2 ml fresh BHI broth media and incubated for one week.

After one week, cases which induced turbidity in culture media were considered as infected cases and then results were analyzed using chi-square and Fisher exact tests.

Results

In this study 40 teeth were evaluated. Teeth were divided into 4 group:

- **Group 1:** included 5 teeth which all of them were turbid after preparation and rinsing with distilled water and after one week incubation (positive control group).
- **Group 2:** included 5 teeth which none of them were turbid after preparation and rinsing with distilled water and autoclaving and after one week incubation (negative control group).
- **Group 3:** included 15 teeth which 3 of them were turbid after preparation and rinsing with 1.3% NaOCl solution protocol (E. faecalis was eliminated in 80% of samples).
- **Group 4:** included 15 teeth which none of them were turbid after preparation and rinsing with MTAD protocol (E. faecalis was eliminated in 100% of samples).

Statistical analysis was not shown significant difference in turbidity between two groups (lack of turbidity was meaning as complete disinfection of tooth and turbidity was considered as dental infection).

Morphological evaluation of colonies from samples which would be turbid in BHI culture media (group 3) and then Gram staining and observing Gram positive cocci as short chain and finally differential test (Trypticase soy Broth plus 6.5% NaCl) confirmed the infection of culture media to Enterococcus faecalis.

Discussion

Microorganism elimination is the main goal of endodontic treatment and biomechanical preparation followed by application of effective irrigant can significantly decrease the microbial population of infected canals.[9]

Enterococcus faecalis is one the most common microorganisms which seen in cases if failure in root treatment [9] and therefore, in this study samples were infected with this microorganism.

Ability of MTAD in complete disinfection of canals was similar to those found by Torabinejad *et al*.[10] and Shabahang *et al*.[11] The cause of ability of MTAD in complete disinfection of canals, elimination of smear layer and ability to penetrate in dental tubules is existence of deoxycycline component. Although there are some studies which indicated the E. faecalis resistance to certain antibiotics such as tetracycline, but the prevalence of these resistant strains is too low. The effectiveness of doxycycline may be due to lower pH, anti-collagenase activity and its ability to attach to dentin and then slow releasing from it.[10,12]

In the study of Torabinejad and colleagues, 50% of samples were infected after preparation of samples by 1.3% NaOCl solution.[10] Results of the present study showed that 20% of the samples were remained infected by using of 1.3% NaOCl solution protocol. Although our findings were important, but no significant differences were detected between two experimental groups.

Different results may be due to different used methods. For instance, Torabinejad *et al*.[12] used initial file no. 10-15 but in our study we used file no. 25 due to higher diameter of premolar teeth canal.

Naturally, with increasing the canal diameter, the penetration power of rinsing to apical parts is increased and therefore its antimicrobial effects and the number of disinfected samples are increased. On the other hand with decrease in canal diameter, the air bobbles were trapped in apical part and prevent the appropriate contact of rinsing material to the area.[13]

Our findings are in disagreement with those found by Krause *et al*.[14] The causes of differences between this two studies can be summarized as follow:

1. Different strains of Enterococcus faecalis in two study
2. The concentration of doxycycline in that study was 10% but this concentration in MTAD is 3%.
3. In the above mentioned study, the MTAD was not used as the final rinsing solution.

4. In the above mentioned study, the teeth deepening step in rinsing solutions was deleted due to simulation of clinical status.
5. In their study protocol, teeth were cut horizontally in 5 mm × 6 mm pieces of bovine tooth and therefore the penetration power of rinsing would be higher in lower root length.
6. Bacteria were collected from dentin shaving.

Results of the present study are also in disagreement with those obtained by Johal and colleagues. In the study of Johal *et al*., antimicrobial effects of MTAD solution as final rinsing solution (first 1.3% NaOCl and then MTAD) and antimicrobial effects of concurrent use of NaOCl and 15% EDTA were evaluated. The culture results of dentin pieces indicated that 50% of samples were remained infected after application of MTAD/1.3% NaOCl. But none of the samples were infected after application of 1.3%NaOCl and 15% EDTA.[15]

The cause of difference between the results of the present study and the above mentioned study is due to difference in studying method. First, for simulation of clinical conditions, the 5 min deepening step in rinsing solutions has not been met. Second, sampling was done immediately after rinsing with respected solutions and the 1 week incubation time was not considered.

In another study which performed by Kamberi *et al*. on the antimicrobial effects of different rinsing solutions (MTAD, 1.5 and 3% NaOCl, EDTA and their combinations), the bactericidal effects of MTAD were higher than NaOCl and EDTA.[16] In the study which performed by Rizvi *et al*. about the evaluation of antimicrobial effects of MTAD, 1 and 5.25% NaOCl, 17% EDTA, and 2% chlorohexidine, it was found that MTAD was the most potent rinsing solution against Enterococcus faecalis and could eliminate this microorganism from canal space.[17] The results of the recent two studies are in line with our findings and demonstrate the superiority of MTAD against other rinsing solution in elimination of Enterococcus faecalis from the canal.

Conclusion

MTAD solution has the high level of antimicrobial properties against Enterococcus faecalis and can be used as final rinsing solution after application of 1.3% NaOCl.

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